The Selenium Metabolite Methylselenol Inhibits the Migration and Invasion Potential of HT1080 Tumor Cells

Huawei Zeng,[1] Mary Briske-Anderson, Joseph P. Idso, and Curtiss D. Hunt

United States Department of Agriculture, Agricultural Research Service, Grand Forks Human Nutrition Research Center, Grand Forks, North Dakota 58202-9034

ABSTRACT There is increasing evidence for the efficacy of certain forms of selenium as cancer-chemopreventive compounds. Methylselenol has been hypothesized to be a critical selenium metabolite for anticancer activity in vivo. To determine whether tumor cell migration, invasion, and cell cycle characteristics are inhibited by methylselenol, we exposed HT1080 cells to methylselenol. Methylselenol was generated with seleno-L-methionine (a substrate for methioninase). Submicromolar methylselenol exposure led to an increase in the G1 and G2 fractions with a concomitant drop in the S-phase, indicating slower cell growth. Furthermore, methylselenol inhibited the migration and invasion rate of the tumor cells by up to 53 and 76%, respectively, when compared with the control tumor cells. Although all cells had increased matrix metalloproteinase (MMP) enzyme activities of pro-MMP-2 and pro-MMP-9, the active form of MMP-2 was decreased in HT1080 cells cultured with methylselenol. In addition, methylselenol increased the protein levels of antimetastasic tissue inhibitor metalloproteinase (TIMP)-1 and TIMP-2. Collectively, these results demonstrate that submicromolar concentrations of methylselenol increase both prometastasis MMP-2 and MMP-9 and antimetastasis TIMP-1 and TIMP-2 expression. The apparent net effect of these changes is the inhibition of pro-MMP-2 activation and carcinogenic potential or activity. J. Nutr. 136: 1528–1532, 2006.

KEY WORDS: • *selenium* • *cell cycle* • *migration* • *invasion* • *cancer*

There is currently much interest in the potential cancer-protective effects of selenium. For example, a recent gene therapy study demonstrated, for the first time, that methioninase expression in tumor cells converts seleno-L-methionine (SeMet)[2] into the selenium metabolite, methylselenol, which has anticancer properties (1). In that study, methylselenol activated the caspase cascade and apoptosis in tumor cells. Furthermore, in vivo SeMet treatment of nude mice bearing tumor cells that express the L-methionine-gamma-lyase (METase) transgene significantly inhibited ascites tumor growth and prolonged host survival (1). The above data provide direct evidence that methylselenol may be responsible for anticancer effects in both cell and whole animal models. These findings offer a new paradigm for cancer gene therapy, and are consistent with previous data on the cancer-protective effect of dietary Se (2–5).

Selenium is an essential trace element currently being considered as a chemopreventive agent for the treatment of cancer (2–8). Epidemiological evidence (2,9) indicates that Se status is inversely associated with cancer risk, and results from intervention studies (2,10) show that high Se intakes effectively reduce the risk of mammary, prostate, lung, colon, and liver cancer (2,9,10). In experimental animals, anticarcinogenic effects have been consistently associated with Se at supranutritional intakes (>1 mg/kg diet) that are at least 10 times those required to prevent clinical signs of deficiency and to support near-maximal tissue activities of selenoenzymes (4,9,11). In this context of cancer chemoprevention, methylselenol has also been hypothesized to be a critical component of the in vivo active Se metabolite pool that induces caspase-mediated apoptosis (3,4).

In view of the reported anticancer properties of methylselenol, in both gene therapy and dietary Se studies (1–10), it is important to characterize the anticarcinogenic mechanism of methylselenol in greater detail. Carcinogenesis is a multistep process that includes tumor initiation, promotion, and progression. In particular, a metastasis, such as tumor migration or invasion, is usually responsible for the deaths of cancer patients (12,13). The results obtained with the recent gene therapy approach clearly demonstrate that methylselenol inhibits tumor growth and prolongs host survival (1). However, whether methylselenol affects the migration and invasion potential of tumor cells remains to be studied. The focus of our study is to determine the mechanism by which methylselenol affects the migration and invasion potential of tumor cells. Our novel finding, that methylselenol modulates the expression of matrix metalloproteinase (MMP) and tissue inhibitor of metalloproteinase (TIMP) and inhibits the migration and invasion potential of tumor cells, provides new insights into the possible mechanisms of the anticancer properties of Se.

[1] To whom correspondence should be addressed. E-mail: hzeng@gfhnrc.ars.usda.gov.

[2] Abbreviations used: FBS, fetal bovine serum; METase, L-methionine-gamma-lyase; MMP, matrix metalloproteinase; SeMet, seleno-L-methionine; TIMP, tissue inhibitor of metalloproteinase.

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MATERIALS AND METHODS

Cell cultures. HT1080 (passage 19) fibrosarcoma cells were obtained from American Type Culture Collection and maintained in DMEM (GIBCO Invitrogen) with 10% fetal bovine serum (FBS; Sigma Chemical). Stock cells were passaged twice weekly at ~<80% confluency [0.25% trypsin (GIBCO), 1 mmol/L EDTA, in Ca-Mg-free Hanks' balanced salt (Sigma)], seeded into new flasks at ~1100–2300 cells/cm^2 (viability was determined by trypan exclusion on hemacytometer counts), and incubated in a humidified chamber at 36.5°C, 5% CO_2. The cultures were tested and found to be mycoplasma free (14). Experiments were performed between passages 23 and 50. Stock cells were grown in a standard medium and supplemented with 10% FBS.

Methylselenol generation. Purified METase of recombinant METase, based on the gene from *Trichomonas vaginalis* produced in *Escherichia coli*, was purchased from Wako Pure Chemical Industries. The enzyme (METase) preparations were reconstituted in PBS, and aliquots were stored at −80°C. For cell treatments, the enzyme substrate seleno-L-methionine (SeMet) (Sigma Chemical) was added to the cell-culture medium and was immediately followed by the addition of METase.

Cell cycle analysis. The cell cycle was analyzed using flow cytometry with propidium iodide staining. HT1080 cells were tryspinsized and washed once with PBS and fixed in 70% (v:v) ethanol at −20°C. After fixation, cells were washed with PBS and stained with 50 mg propidium iodide/L, containing 6000 U ribonuclease A/L (RNAase A/L). The DNA contents of cells were determined by flow cytometry. Data were stored as list mode files of at least 10,000 single cell events and analyzed by EPICS profile II and ModFit LT software (Coulter).

Cell invasion and motility assays. The invasion and motility of methyselenol-treated HT1080 cells were determined using BD BioCoat Matrigel Invasion Chambers, Falcon Cell Culture Inserts (8-μm pore PET membrane), and Falcon TC Companion Plates (24-well plate, BD Biosciences). Invading cells (but not migrating cells) required extracellular matrix degradation, and matrigel was designed to simulate extracellular matrix. Matrigel-coated inserts (for invasion assay) and uncoated inserts (for migration assay) were suspended in DMEM and rehydrated for 2 h in a humidified, 36.5°C, 5% CO_2 incubator, according to the manufacturer's instructions. Hydrated inserts were transferred to wells containing 0.75 mL of DMEM supplemented with 5% FBS. The control and methyselenol-treated cells were harvested by trypsinization, washed with FBS supplemented DMEM, centrifuged at 110 × *g* for 5 min at 4°C, resuspended in DMEM, and seeded into matrigel and uncoated inserts at 15,000 cells in 0.5 mL DMEM. The chambers were incubated for 14–16 h in a humidified incubator at 36.5°C, 5% CO_2. After incubation, the upper surfaces of the membranes were scrubbed with a cotton tip swab to remove noninvading or migrating cells. The invading and migrating cells on the lower surfaces of the membranes were fixed and stained with a Wright-Giemsa quick stain (Camco Stain Pak, Cambridge Diagnostic Products). Dry membranes were mounted in immersion oil on microscope slides and cover slipped. Random fields (10/membrane), representing all regions of the membrane, were photographed at 40× magnification.

TIMP assay and MMP zymography. The methyselenol-treated HT1080 cells were rinsed and incubated for 24 h in the presence of serum and Se-free DMEM for 24 h. Conditioned media were then collected, held on ice, centrifuged at 110 × *g* for 5 min at 4°C to pellet any nonadherent cells, and stored at −80°C until analysis. The conditioned media were assayed for TIMP-1 and TIMP-2 (Chemicon) levels by specific enzyme-linked immunosorbent assay and read at 492 nm and 450 nm, respectively, on a Spectra Max 190 reader (Molecular Devices). The MMP-2 and MMP-9 enzyme activities of conditioned media were separated by electrophoresis on 10% zymogram (gelatin) gels (Invitrogen) and could then be visualized as clear bands against a dark background with Coomassie Blue staining. The enzyme signals were quantified by the UVP Bioimaging Systems.

Statistical analysis. Values are means ± SD. The data were analyzed by 1-way ANOVA using Proc Mixed in SAS, version 9.1 (SAS Institute) with experiment included as a blocking factor. Experimental variables that exhibited nonhomogeneous variances among groups were allowed to be unequal, and Satterthwaite's approximate degrees of freedom were used in Proc Mixed. Statistically significant treatments were compared with controls using Dunnett's multiple comparisons. Differences were considered significant at $P < 0.05$.

RESULTS

HT1080 cell growth and cell cycle progression. The cell growth rate was inhibited by 16.6, 32.6, and 51.7%, respectively, in the cells treated with methylselenol generated by 1.25, 2.5, or 5 μmol/L SeMet when compared with 0 μmol/L SeMet in the presence of METase (40 U/L)-treated medium (**Table 1**). Similarly, G1 phase cell distribution was increased by 28.8 and 42.3%, respectively; G1+ G2 phase cell distribution was increased by 25.7 and 41.1%, respectively; S phase cell distribution decreased by 32.5 and 54.2%, respectively, in the cells treated with methylselenol generated by 2.5 or 5 μmol/L SeMet in the presence of METase (40 U/L)-treated medium (Table 1). In contrast, cell growth and cell cycle progression in cells treated with 5 μmol/L SeMet alone, or METase (40 U/L) alone, did not differ (Table 1).

Effect of methylselenol treatment on migration and invasion of HT1080 cells. The tumor cell migration rate decreased by 25.7 and 53.2%, respectively, in the cells treated with methylselenol generated by 2.5 or 5 μmol/L SeMet when compared with 0 μmol/L SeMet in the presence of METase (40 U/L)-treated medium (**Table 2**). Similarly, the tumor cell invasion rate decreased by 34.9, 56.9, and 76.3%, respectively, in the cells treated with methylselenol generated by 1.25, 2.5, or 5 μmol/L SeMet in the presence of METase (40 U/L)-treated medium (Table 2). In contrast, tumor cell migration and invasion rate in the cells treated with 5 μmol/L

TABLE 1

Effect of methylselenol, generated by incubating 40 U/L METase for 16 h with 0, 1.25, 2.5, or 5 μmol/L SeMet, on HT 1080 cell growth and cycle phase distributions[1]

SeMet, *μmol/L*	METase, *40 U/L*	Cell growth, *million cells/L*	Phase-specific cells/total detected cells, %			
			G1 phase	S phase	G2 phase	G1 + G2 phase
5	−	318.9 ± 13.5	48.2 ± 1.0	43.2 ± 1.0	8.2 ± 0.3	56.4 ± 0.9
0	+	320.1 ± 17.9	48.9 ± 0.4	42.4 ± 1.3	8.3 ± 0.6	57.2 ± 1.1
1.25	+	266.9 ± 17.7*	51.7 ± 2.3	40.0 ± 3.4	8.4 ± 1.2	60.1 ± 3.4
2.5	+	215.8 ± 32.3*	63.0 ± 5.0*	28.6 ± 5.5**	8.9 ± 1.3	71.9 ± 4.8**
5	+	154.7 ± 41.0**	69.6 ± 7.9**	19.4 ± 6.2**	11.1 ± 3.9	80.7 ± 6.2**

[1] Values are means ± SD, $n = 4$.

[2] Asterisks indicate different from control (0 μmol/L SeMet), *$P < 0.05$; **$P < 0.01$.

TABLE 2

The effect of methylselenol, generated by incubating 40 U/L METase for 16 h with 0, 1.25, 2.5, or 5 μmol/L SeMet, on the migration, invasion, and TIMP-1 and TIMP-2 protein levels of HT 1080 cells[1]

SeMet	METase	Migration cells	Invasion cells	TIMP-1	TIMP-2
μmol/L	*40 U/L*	*n/membrane*		*ng/40,000 cells*	
5	−	602.5 ± 16	447.3 ± 28	7.2 ± 1.7	14.8 ± 1.6
0	+	608.3 ± 29	454.8 ± 39	7.3 ± 0.6	14.6 ± 0.7
1.25	+	591.2 ± 65	296.0 ± 85*	9.0 ± 1.9	16.8 ± 1.2*
2.5	+	451.8 ± 98*	196.0 ± 23**	12.8 ± 1.5**	19.0 ± 1.5**
5	+	284.7 ± 20**	107.7 ± 22**	34.6 ± 2.8**	41.0 ± 12.4**

[1] Values are means ± SD, $n = 3$.
[2] Asterisks indicate different from control (0 μmol/L SeMet), *$P < 0.05$; **$P < 0.001$.

SeMet alone, or METase (40 U/L) alone, did not differ (Table 2).

Effect of methylselenol treatment on TIMP-1 and TIMP-2 protein levels. The TIMP-1 protein level increased by 75.3 and 374.0%, respectively, in the cells treated with methylselenol generated by 2.5 or 5 μmol/L SeMet when compared with 0 μmol/L SeMet in the presence of METase (40 U/L)-treated medium (Table 2). Similarly, the TIMP-2 protein level increased by 15.1, 30.1, and 180.8%, respectively, in the cells treated with methylselenol generated by 1.25, 2.5, or 5 μmol/L SeMet in the presence of METase (40 U/L)-treated medium (Table 2). In contrast, the TIMP-1 and TIMP-2 levels of the cells treated with 5 μmol/L SeMet alone, or METase (40 U/L) alone, did not differ (Table 2).

Effect of methylselenol treatment on MMP-2 and -9 enzyme activity. The enzyme activity of latent forms of MMP-2 increased by 108.3, 142.3, and 207.3%, respectively, in the cells treated with methylselenol generated by 1.25, 2.5, or 5 μmol/L SeMet when compared with 0 μmol/L SeMet, in the presence of METase (40 U/L)-treated medium (**Fig. 1**, **Table 3**). Similarly, the enzyme activity of latent forms of MMP-9 increased by 58.3 and 95.6%, respectively, in the cells treated with methylselenol generated by 2.5 or 5 μmol/L SeMet in the presence of METase (40 U/L)-treated medium (Fig. 1, Table 3). However, the enzyme activity of the active form of MMP-2 decreased by 45.4, 78.3, and 95.2% in the cells treated with methylselenol generated by incubating METase (40 U/L) with 1.25, 2.5, or 5 μmol/L SeMet (Fig. 1, Table 3). In contrast, enzyme activity of MMP-2, MMP-9, and the active form of MMP-2 did not differ in the cells treated with 5 μmol/L of SeMet alone or METase (40 U/L) alone (Fig. 1, Table 3).

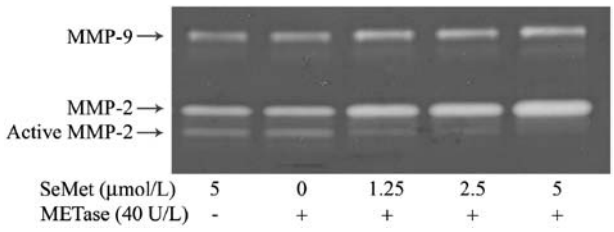

FIGURE 1 Effect of methylselenol, generated by incubating 40 U/L METase for 16 h with 0, 1.25, 2.5, or 5 μmol/L SeMet, on HT 1080 cell growth and MMP-2 and MMP-9 enzyme activities. Representative gelatin zymography showed an increase of MMP-2 and MMP-9 signals but a decrease of active MMP-2 signals in the SeMet/METase-treated cells.

DISCUSSION

The mechanisms underlying the anticancer activity of Se are not fully understood, but animal and cell culture models have yielded much insight. Of particular significance are recent gene therapy data that provide the first direct evidence, to our knowledge, that methylselenol may be responsible for anticancer effects (1). Several lines of evidence implicate methylselenol as the active in vivo Se metabolite pool for anticarcinogenic effects associated with supplements and high Se intakes (3,4,11). Previous studies suggest the potential antiangiogenic effect of methylselenol in the chemoprevention of cancer (3). Angiogenesis is obligatory for early lesion growth and progression as well as metastasis (15). Malignant cancers arise from preexisting benign tumors through multiple steps (16). Cancer cell-matrix interaction is a critical step that promotes cell migration, proliferation, and extracellular matrix degradation (17,18). The proteolytic degradation of extracellular matrix is a critical event during tumor invasion and metastases. Although the breakdown of the basement membrane is achieved by several matrix metalloproteinases (MMPs), MMP-2 and MMP-9 appear to be the most important for basement membrane type IV collagen degradation (16–19). Similar to various malignant tumors, HT1080 cells express MMP-2 and MMP-9 at a high level (20). Thus, the study of the effects of methylselenol on HT1080 cell migration and invasion will further our understanding of functional roles of methylselenol in invasive cancer cells.

TABLE 3

Effect of methylselenol, generated by incubating 40 U/L METase for 16 h with 0, 1.25, 2.5 or 5 μmol/L SeMet, on MMP-2 and MMP-9 enzyme activity of HT 1080 cells[1]

SeMet	METase	MMP-2 activity	MMP-9 activity	Active MMP-2 activity
μmol/L	*40 U/L*		*Intensity units*	
5	−	1538 ± 516	995 ± 161	243 ± 81
0	+	1427 ± 451	997 ± 323	249 ± 41
1.25	+	2972 ± 684*	1379 ± 148	136 ± 36*
2.5	+	3457 ± 842*	1578 ± 162*	54 ± 15**
5	+	4385 ± 648**	1950 ± 424**	12 ± 13**

[1] Values are means ± SD, $n = 3$.
[2] Asterisks indicate different from control (0 μmol/L SeMet), *$P < 0.05$; **$P < 0.001$.

The method of generating methylselenol in cell-culture medium by incubating METase with seleno-L-methionine (SeMet) as a substrate has been well documented (1,21,22). In several different culture media, methylselenol, generated by incubating METase with seleno-L-methionine (SeMet) but not L-methionine (Met) as a substrate, has been shown to be responsible for such anticarcinogenic effects as cell cycle arrest and apoptosis (1,22). Based on our experience, there were no detectable effects on cell growth, cycle, migration, invasion, or on TIMP and MMP activity, when incubating METase with additional 5 μmol/L L-methionine (Met) into our DMEM cell-culture medium, which contained 201.1 μmol/L L-methionine (endogenous concentration as standard medium composition). Furthermore, our data demonstrated that cell growth, cycle, migration, invasion, or TIMP and MMP activity did not differ in the cells treated with 5 μmol/L SeMet alone or METase (40 U/L) alone. Therefore, methylselenol generation and its detectable effects in our experimental condition were highly specific.

Our data showed that methylselenol inhibits cell growth by increasing the G1 cell fractions with a concomitant drop in the S-phase. This observation is consistent with and complements previous gene-therapy data indicating that an increasing sub-G0 cell population and a strong bystander (cytotoxic) effect occurs because of a methylselenol release from SeMet by tumor cells with the adenoviral-delivered METase gene, and that methylselenol damages the mitochondria via oxidative stress and causes cytochrome c release into cytosol, thereby activating the caspase cascade and apoptosis (1).

Importantly, we showed that methylselenol inhibited the migration and invasion rate of HT1080 tumor cells and increased enzyme activities of pro-MMP-2 and pro-MMP-9 in HT1080 tumor cells. Both pro-MMP-2 and pro-MMP-9 have been reported to be elevated in human tumor tissues (16). The increase in the expression of pro-MMP-2 and pro-MMP-9 appears paradoxical to the anticancer effect of methylselenol. However, a further study demonstrated that a significant correlation between the metastatic phenotype and MMP-2 and MMP-9 concentrations was noted only with the activated forms (16), and that their activities are regulated by natural specific inhibitors, i.e., the tissue inhibitors of metalloproteinase (TIMPs) (23). Therefore, the ratio between MMPs and TIMPs may influence the metastatic phenotype, as well as other physiologic processes. The significant increases in protein levels of TIMP-2 and TIMP-1 in our study suggest at least 2 important molecular roles of methylselenol. First, the upregulation of TIMP-2 and TIMP-1 suppresses the activation of pro-MMP-2 and pro-MMP-9, respectively, and may partly account for the basis of the inhibitory effect of methylselenol treatment on the migration and invasion of tumor cells. This is supported by the fact that few detectable active MMP-2 forms were found in methylselenol treated cells, even though pro-MMP-2 and pro-MMP-9 were also upregulated. Second, TIMP-2 may inhibit tumorigenesis in a MMP-independent pathway. The epidermal growth factor receptor is reported to be highly expressed in human cancers but is found at lower levels in normal tissues, and TIMP-2 can directly suppress the activation of a mitogenic response by inhibiting tyrosine kinase-type receptor activation (24,25).

Our novel finding on the expression of MMPs, TIMPs, and the inhibitory effect of methylselenol treatment on the migration and invasion of tumor cells may explain, in part, the earlier observation (1) that SeMet treatment of nude mice, bearing tumors cells expressing the METase transgene, significantly inhibits ascites tumor growth and prolongs host survival. Our data also suggest that a submicromolar amount of methylselenol (21,22) should be sufficient to inhibit the migration and invasion of HT1080 tumor cells. The mean plasma Se concentration of subjects without Se supplementation has been documented to be ~1.5 μmol/L; however, supplementation (at 200 μg/d as selenized yeast) associated with a >50% reduction in the risk of prostate, colon, and lung cancer, brings the mean Se level to ~2.4 μmol/L in recent human trials (10). With high Se intake, the extra Se that is not used for selenoprotein synthesis is then used to incorporate selenomethionine into plasma proteins (albumin, immunoglobulins, etc.) and/or to enrich the methylselenol pool by the methylation pathway (4,11). Although it remains to be determined whether in vivo methylselenol can reach submicromolar levels (21,22), the high cell proliferation rate in the local environment of the tumor, as compared with normal tissue cells, is likely to increase circulating and tissue levels of Se methylselenol through blood-borne Se, which may directly inhibit tumor cell migration and invasion.

In summary, our data demonstrate that methylselenol increases the expression of both prometastatic genes, MMP-2 and MMP-9, and antimetastatic genes, TIMP-1 and TIMP-2, and that the net effect of these increases is the inhibition of pro-MMP-2 activation and of tumor cell migration and invasion capacity.

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